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A Phase I Study of IMP321 and Gemcitabine as the Front-line Therapy in Patients with Advanced Pancreatic Adenocarcinoma

Andrea Wang-Gillam^{1,4,*}, Stacey Plambeck-Suess^{2,*}, Peter Goedegebuure^{2,4}, Peter O Simon³, Jonathan B Mitchem², John R Hornick², Steven Sorscher^{1,4}, Joel Picus^{1,4}, Rama Suresh^{1,4}, Albert C Lockhart^{1,4}, Benjamin Tan^{1,4}, and Williams Hawkins^{2,4}

¹Division of Oncology, Department of Medicine, Washington University School of Medicine, St. Louis, MO

²Department of Surgery, Washington University School of Medicine, St. Louis, MO

³Vascular and Interventional Radiology, University of Virginia Health System, Charlottesville, Virginia

⁴Siteman Cancer Center, St. Louis, MO

Abstract

Purpose—This phase I study was conducted to determine the safety profile and maximum tolerated dose (MTD) of IMP321, a soluble lymphocyte activation gene-3 (LAG-3) Ig fusion protein and MHC Class II agonist, combined with gemcitabine in patients with advanced pancreatic adenocarcinoma.

Patients and methods—Patients with advanced pancreatic adenocarcinoma were treated with gemcitabine (1,000 mg/m²)(level 1), gemcitabine (1,000 mg/m²) plus IMP 321 at 0.5 mg (level 2) and 2.0 mg (level 3), respectively. Safety, toxicity, and immunological markers at baseline and post treatment were assessed.

Results—A total of 18 patients were enrolled to the study, and 17 were evaluable for toxicity. None of the 6 patients who received 0.5 mg IMP321 experienced IMP321-related adverse events. Of the 5 patients who received IMP321 at the 2 mg dose level, 1 experienced rash, 1 reported hot flashes and 2 had mild pain at the injection sites. No severe adverse events previously attributed to IMP321 were observed. No significant differences were observed when comparing pre- and post-treatment levels of monocytes (CD11b+CD14+), conventional dendritic cells (CD11c+) or T cell subsets (CD4, CD8).

Conclusions—IMP321 in combination with gemcitabine is a well-tolerated regimen. IMP321 did not result in any severe adverse events. No incremental activity observed for the additional IMP 321 to gemcitabine at the dose levels evaluated, likely due to sub-optimal dosing. Immunological markers suggested that higher dose levels of IMP321 are needed for future clinical studies.

Keywords

IMP321; LAG-3; front-line therapy; chemo-immunotherapy; pancreatic adenocarcinoma

Corresponding author: William G. Hawkins MD, FACS, Associate Professor of Surgery, Department of Surgery, Washington University School of Medicine, St. Louis, MO 63110, hawkinsw@wustl.edu.

*Denotes equal contribution

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The authors declare that they have no conflict of interest.

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related mortality in the United States, resulting in approximately 37,000 deaths in 2011 [1]. Surgery is the only curative therapy; however, about 80% of patients are not surgical candidates at the time of diagnosis, and their treatment options are largely limited to palliative chemotherapy and radiation. Over the last decade, the 5-year overall survival for patients with advanced pancreatic cancer has remained less than 5% [1].

Gemcitabine has been a cornerstone regimen for patients with pancreatic cancer since 1997 after a phase III study demonstrated a modest survival advantage and better disease-related symptom control in patients treated with gemcitabine when compared to patients treated with 5-FU [2]. Since then, various regimens using gemcitabine in combination have been tested in the clinical setting; so far, only gemcitabine plus erlotinib has resulted in a moderate survival benefit when compared to gemcitabine alone, gaining approval from the Food Drug Administration (FDA). However, the clinical value of erlotinib has been questioned given its minimal improvement in median overall survival (OS) (6.24 months vs. 5.91 months) [3].

Cancer immunotherapy has been an active area of basic cancer research for decades. Recently, the immunological approach has become extremely promising for solid tumors based on survival benefits observed in the clinical setting [4, 5]. Additionally, the so-called “hallmarks of cancer” posited by Drs. Hanahan and Weinberg, recently were revised to include immune suppression [6]. Current clinical studies for pancreatic cancer emphasize immune activation and/or attempt to modulate mechanisms of immune suppression [7]. In pancreatic cancer in particular, immune suppression is prominent through an increased prevalence of myeloid-derived suppressor cells (MDSC) including suppressor macrophages [8, 9] and regulatory T-cells (Treg) that correlate with poor prognosis [10-12]. While MDSC can suppress antitumor immunity through a variety of mechanisms [8], Tregs induce immune tolerance by suppressing immune responses against self and non-self antigens [10]. Thus MDSC and Treg play a critical role in the immune response to malignancies.

The lymphocyte activation gene-3 (LAG-3 or CD223) is an important T cell regulator [13]. As a transmembrane protein that forms dimers at the surface of activated T cells, LAG-3 has a high affinity for major histocompatibility complex (MHC) class II molecules expressed on antigen presenting cells (APC) [13]. The LAG-3 - MHC class II interaction promotes the activation and maturation of human dendritic cells (DC), resulting in DC chemokine and cytokine secretion [13]. Additionally, LAG-3 has been shown to elicit a tumor-specific cytotoxic lymphocyte (CTL) response and a CD4⁺ TH₁ response when administered as an adjuvant with tumor cells [14, 15]. When activated, LAG-3 is a T-cell receptor (TCR)-associated molecule that negatively regulates TCR-mediated signaling in effector T cells in a fashion analogous to the cytotoxic T lymphocyte associated protein 4 (CTLA-4) [16-19]. This LAG-3 and TCR signaling pathway is necessary for Treg suppressor activity [20, 21]; therefore, exogenous (or soluble) LAG-3 may potentially interfere with the LAG-3 activation of Treg and block Treg-mediated tolerance when used at high dose levels.

Soluble LAG-3 protein can be detected in human serum. In patients with breast cancer, higher soluble LAG-3 (sLAG-3) levels were associated with better survival [22]. The potential therapeutic benefit of sLAG-3 has led to the development of IMP321, a recombinant form of sLAG-3 (Immutep S.A., Orsay, France). IMP321 consists of an extracellular portion of human LAG-3 fused to the F_c fraction of human IgG1, thus creating a stable dimeric structure. IMP321 binds to MHC II on the surface of APCs and promotes activation and maturation of human DCs, resulting in chemokine and cytokine secretion

which has been shown to have strong immunostimulatory effects in mice immunized with particulate or soluble protein antigens [23].

The safety of IMP321 was previously tested in 2005 in 108 healthy volunteers either alone or with immunization with two antigens which have been used widely in human vaccination trials [24, 25]; excellent local and systemic tolerability was observed [26]. We hypothesize that the combination of IMP321 and gemcitabine may induce or amplify pre-existing cell-mediated immune responses by stimulating APCs in the presence of cytotoxic therapy.

Methods

Study design

This was a phase I, open-label, dose escalation study of IMP321 combined with gemcitabine as the front-line therapy in patients with newly diagnosed advanced pancreatic cancer. The first dose level was defined as 1,000 mg/m² gemcitabine administered as a single agent intravenously (IV) every week for 7 out of 8 weeks. This group 1 cohort was added to the protocol based on the recommendation of the FDA. The Level 2 cohort received 0.5 mg IMP321 subcutaneously (s.c.) on days 2 and 16 while receiving 1,000 mg/m² gemcitabine every week for 3 of 4 weeks. The Level 3 cohort was similar to Group 2 except these participants received a 2.0 mg dose of IMP32. Every 4 weeks was considered one cycle. Imaging studies were performed on patients prior to enrollment in the trial and were then repeated every two treatment cycles. Treatment efficacy was assessed by the Response Evaluation Criteria in Solid Tumor (RECIST) 1.1. The maximal treatment duration was 6 cycles. IMP321 at 6 mg and 12 mg was planned but not performed because the manufacturer was unable to produce further product. The dose-limiting toxicity (DLT) was defined as follows: any Grade 3 or Grade 4 non-hematological toxicity with the exceptions of Grade 3 nausea or vomiting, and Grade 3 hepatic toxicity which returned to Grade 1 prior to the next treatment course. The hematological DLT was defined as Grade 4 neutropenia greater than 7 days duration, Grade 4 anemia or thrombocytopenia that required transfusion therapy on greater than 2 occasions in 7 days or a delay greater or equal to 14 days between treatment cycles. Any toxicity requiring an additional 2 weeks delay between cycles was considered to be a DLT. DLT was not limited to the first treatment cycle, and any subject experiencing Grade 4 cumulative toxicities after the first cycle was considered to experience DLT. Toxicity was graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0. Subjects were not enrolled to the next dose level until all subjects on the previous dose level had completed one full cycle. The MTD was defined as the dose level immediately below the dose level at which 2 patients of a cohort (6 patients) experienced DLTs.

Patient population

Patients with newly diagnosed histologically or cytologically confirmed unresectable pancreatic adenocarcinoma were eligible for this study. The eligibility criteria were defined as follows: measurable or evaluable disease defined by RECIST criteria; > 18 years old; Karnofsky Performance Status (KPS) ≥ 70; life expectancy >12 weeks; adequate bone marrow functions defined as an absolute neutrophil count > 1,500/mm³, platelet count >100,000/mm³ and hemoglobin >10 g/dL; adequate renal function defined as serum creatinine ≤ 2.0 mg/dL or creatinine clearance ≥ 60 mL/min/1.73 m²; adequate hepatic function defined as total bilirubin ≤ 1.5 X the institutional upper limit normal value (ULN) and aspartate aminotransferase (AST) ≤ 2X the ULN. Patients must have signed the informed consent form. Patients with prior history of chemotherapy for pancreatic cancer, or active autoimmune disease requiring management with immunosuppressive drugs were excluded from this study.

Drug Administration

Patients in the first dose level (gemcitabine only) received 1,000mg/m² IV gemcitabine over a 30 minute period every week for 7 of 8 weeks. Patients participating in the combination drug regimen cohorts (levels 2 and 3) received 1,000 mg/m² IV gemcitabine over a 30 minute period every week for 3 of 4 weeks. Level 2 and 3 patients also received IMP321 administered s.c. with the first dose on the anterior surface of either the left or right thigh on day 2, and the subsequent dose on day 16 delivered on the contralateral thigh. IMP321 at 0.5 mg and 2 mg were given for dose levels 2 and level 3, respectively. Subjects who received IMP321 were monitored for 1 hour following drug administration. Dose modifications for gemcitabine were made according to the manufacturer's recommendations.

Immunological Assays

Flow cytometric analysis using peripheral blood mononuclear cells (PBMC) from patients with pancreatic adenocarcinoma was performed as follows. Peripheral blood samples were collected from study subjects in vacuum tubes containing heparin (BD Biosciences; San Jose, CA) and processed immediately. Cells were isolated by Ficoll-density centrifugation and frozen in dimethyl sulfoxide (DMSO) with 10% fetal bovine serum (FBS). When both pre – and post treatment blood samples were collected, PBMC were thawed using CTL wash (Cellular Technology Ltd, Shaker Heights, OH), washed, and stained with antibodies including CD45, CD11b, CD11c, CD4, CD8, CD14, CD15, CD80, CD86, CD123, and MHC class II, according to the manufacturer's instructions (BD Biosciences). Analysis was immediately performed on a LSR II flow cytometer. Corresponding isotope controls yielded no significant staining. All results were analyzed using FlowJo software (Tree Star Inc, Ashland, OR) and are expressed as a percentage of CD45⁺ cells.

Results

General

From February to August 2009, a total of 18 patients were consented to the study. One patient withdrew consent before receiving any treatment. Seventeen were considered to be evaluable for toxicity, as defined by those receiving at least one dose of chemotherapy. Demographic information is shown in Table 1. The median age of all patients was 63.5 years. Out of 17 patients, 9 (53%) were male, and 12 (71%) were Caucasian. The majority (71%) of patients had a KPS of 70-80. Thirteen (76.4%) had metastatic pancreatic cancer while 4 had locally advanced disease (2 in dose level 1 group, 2 in level 2, and 1 in level 3). Eight patients had tumors in the head of the pancreas. Six patients received gemcitabine only (level 1); 6 received gemcitabine and IMP321 at the 0.5 mg dose level (level 2), and 5 received gemcitabine plus IMP321 at the 2 mg dose level (level 3).

Safety and Toxicity

The adverse events related to IMP321 are summarized in Table 2. None of the 6 patients who received 0.5 mg IMP321 experienced IMP321-related adverse events. Of the 5 patients who received IMP321 at the 2 mg dose level, 1 experienced rash, 1 reported hot flashes, and 2 had mild pain at the injection sites. Two patients in this cohort experienced DLTs, and both DLTs (grade 3 pneumonitis and grade 3 abdominal pain) were considered to be gemcitabine-related. No severe adverse events attributable to IMP321 were observed.

The reasons for treatment discontinuation of subjects are shown in Table 3. Of the 17 evaluable patients, 5 (29.4%) were taken off study due to disease progression, 4 were taken off study due to gemcitabine-related adverse events, and 8 (47.1%) completed a total of 6 treatment cycles.

Antitumor activity

For the gemcitabine only group (Level 1), 5 of 6 patients had stable disease (SD). The median time-to-progression (TTP) and overall survival (OS) were 44.5 and 72.7 weeks, respectively. For patients receiving the level 2 dose, 2 of 6 experienced SD. The median TTP and OS were 8.8 and 24.5 weeks, respectively. One patient with metastatic pancreatic cancer in this cohort had SD for 33 weeks and OS for 101 weeks. Five patients received the level 3 dose, and 2 with DLTs were not evaluable for treatment efficacy as no imaging studies were performed on them. All 3 evaluable patients had SD. Two in this cohort completed 6 cycles of treatment. The median TTP and OS were 23.0 and 28.0 weeks, respectively.

Immunological analysis

As LAG-3 reportedly promotes antigen presentation and T cell activation, PBMC from patients were subjected to immunophenotypic analysis before and after completion of treatment. In addition to subset analysis, expression levels of cell surface markers indicative of activation/maturation were assessed. Overall, no significant differences were observed when comparing pre-treatment with post-treatment levels of monocytes (CD11b⁺CD14⁺), conventional dendritic cells (CD11c⁺) or T cell subsets (CD4⁺, CD8⁺). This was observed when comparing all patients as one group; when broken down by cohort, or when comparing patients with the same clinical response, i.e. SD or progressive disease (PD). Pre-treatment and post-treatment prevalence did not significantly differ between various subgroups of patients such as patient in level 2 versus 3 or between SD and PD patients (Figure 1). Likewise, when comparing expression levels of MHC class II (HLA-DR), CD80, and CD86, no significant differences were noted within subgroups of patients or within patients comparing pre – versus post-treatment.

Discussion

IMP321 in combination with gemcitabine was a tolerable regimen. There were two DLTs when IMP321 was given at the 2 mg dose level in combination with gemcitabine, and both were attributed to gemcitabine. The FDA mandated reevaluation of our trial after the first two dose levels were completed. Toxicity data was shared with the FDA and the safety profile was considered acceptable. Approval was given for additional study of IMP321 at higher and potentially more effective doses of in combination with gemcitabine. Further studies are under consideration and will be determined based on corporate goals and resource availability. Escalation beyond the 2mg level was not performed in this study due to the lack of available experimental drug. When interpreting the data from this study, it is important to note that the FDA mandated a gemcitabine cohort (Level 1) which demonstrated an unusually long survival and was not comparable with the historical data for gemcitabine alone [2].

IMP321 has previously been shown to increase T cell responses and vaccine immunogenicity in a proof-of-concept phase I study [24, 25]. Additionally, a preclinical study demonstrated that the presence of soluble LAG-3 resulted in renal cell cancer (RCC) rejection in mice [15], leading to a phase I study of IMP321 in patients with advanced renal cell carcinoma [26]. IMP321 at 0.05, 0.25, 1.25, 6.25 and 30 mg s.c biweekly doses was assessed, and no clinically significant treatment-related adverse events were reported at any dose levels. Based on the pharmacokinetic analysis, plasma concentrations of IMP321 were minimally detectable in the 1.25 mg dose group [26]. According to the pharmacodynamic analysis, IMP321 increased the percentage of activated CD8⁺ T cells in higher dose groups (6.25 mg and 30 mg). Furthermore, although there was no objective response in the 21 subjects that participated in this phase I trial, progression-free-survival was significantly

better in the high dose group (> 6 mg) [26]. Unfortunately, the data of this phase I study was published after our phase I study launched in the United States, and the IMP321 dose levels that we used (0.5 mg and 2mg) were considered to be suboptimal based on the pharmacodynamic analysis of the prior RCC trial.

In addition to the above trial in RCC patients, IMP321 in combination with paclitaxel was tested in patients with breast cancer [27] in the first chemo-immunotherapy trial with this drug. Chemotherapy can increase antigen release by inducing tumor cell apoptosis, and IMP321 leads to APC activation and activation and expansion of $CD8^+$ T cells [27]. Three doses of IMP321 (0.25 mg, 1.25 mg and 6.25 mg) in combination with paclitaxel were administered in this study. There were no significant IMP321-related adverse events [27]. The 6.25 mg IMP321 dose was found to activate monocytes when given repeatedly over 6 months [27]. Monocyte activation in the blood at 6 months was positively correlated with tumor regression [27]. The gain of function in this major APC subset, i.e. the monocytes, was already observed at 3 months and then strongly reinforced at 6 months [27]. Thus IMP321 in the 6 mg dose range should be given repeatedly as an APC activator to induce its primary pharmacodynamic effect. In the present study only patients who are first stabilized with gemcitabine and therefore have received IMP321 for more than 3 months could theoretically benefit from the immunotherapy.

Our study along with other studies demonstrates a good safety profile of IMP321 either as a single agent therapy or in combination with chemotherapy, warranting the exploration of higher doses of IMP321 in future clinical trials.

In our study, two cohorts of IMP321 plus gemcitabine did not show treatment efficacy. This is likely due to the low IMP321 doses. Treatment response was seen in patients received the lowest dose (0.25mg) of IMP321 in the phase I trial of IMP321 and paclitaxel in breast cancer; however, the treatment benefit is probably due to the paclitaxel effect [27].

In terms of pharmacodynamic analysis of this study, levels of monocytes ($CD11b^+CD14^+$), conventional dendritic cells ($CD11c^+$) and T cell subsets ($CD4^+$, $CD8^+$) were not changed between pre- and post-treatment. This observation may also be related to the low doses of IMP321 used in this study, as previous studies did not reveal any significant impact on $CD8^+$ activation by monocytes when IMP321 was given at the low dose levels [26, 27]. IMP321 at 6.25 mg, however, was found to activate monocytes when given repeatedly over 6 months [27], and this dose appears to be tolerable; therefore it should be considered for future chemo-immunotherapy trials.

Chemotherapy regimens for patients with pancreatic cancer have recently gone through revolutionary changes. A phase III randomized trial has demonstrated a superior overall survival in patients with metastatic pancreatic cancer when they were treated with FOLFIRINOX (5-FU, leucovorin, oxaliplatin and irinotecan) compared with gemcitabine (median OS 11.6 months vs. 6.8 months; HR: 0.57; $p<0.0001$) [28]. The significant improvement of OS with FOLFIRINOX has led to the further evaluation of its benefit in locally advanced and resectable pancreatic cancer. The toxicity associated with FOLFIRINOX has made combination with other cytotoxic therapies challenging. In light of the lack of clinically adverse events observed with IMP321, it is a reasonable to explore the combination of IMP321 and FOLFIRINOX in patients with pancreatic cancer. Additionally, combination with other immune modulating therapies could be explored. Various strategies are being tested in clinical trials including specific T cells [7]. To increase efficacy, these strategies can be combined with strategies that modulate immune suppressor cells or T cell regulatory networks such as CTLA-4 and Programmed Death-1 [7,8]. We anticipate that

studies of IMP321 combined with either chemotherapy or immunotherapy will flourish soon.

Conclusions

This is the first investigation of IMP321 in the United States. We have demonstrated that low dose IMP321 is safe in combination with gemcitabine chemotherapy. We did not observe a beneficial clinical effect or positive basic science correlation at the doses tested in this phase I trial. We do believe that further evaluation of IMP321 is warranted in patients with pancreatic cancer given that the doses utilized in this study were lower than what has previously been shown to have an effect in other malignancies. We recommend doses greater than 6 mg be considered in future U.S. trials based on the safety profile and efficacy observed in recent European trials.

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Figure 1. Comparison of immunological markers between dose level groups 2 and 3
Cumulative flow cytometry results using PBMC from patients before (BT) and after treatment (OT). In addition to total number of MHC class II positive (panels a and d), the prevalence of myeloid DC was determined by assessing the percentage of CD14-CD123-CD11c+ (panels b and e), and expression of CD80 and CD86 on CD11c+ cells (panels c and f). Panels a-c represent data from level 2 patients, and panels d-f represent level 3 patients.

Table 1

Baseline clinical and demographic characteristics

Characteristic	Patients (<i>N</i> = 17)	
	No.	%
Gender		
Male	9	53
Female	8	47
Age, years		
Median	63.5	
Range	36 - 90	
Ethnicity		
White	12	71
Non-White	5	29
Karnofsky performance status (KPS)		
70-80	12	71
90-100	5	20
Tumor Stage		
Locally advanced	4	24
Metastatic	13	76
Tumor Site		
Head	8	47
Non-Head	9	53

Table 2

A Summary of all adverse events related to IMP321.

Toxicity	Dose Level					
	Level 2 (n = 6)		Level 3 (n = 5)		Total (n = 11)	
	All	Grade 3	All	Grade 3	All	Grade 3
Dermatology/Skin						
Rash/desquamation	0	0	1	0	1	0
Endocrine						
Hot Flashes	0	0	1	0	1	0
Pain - Injection Site	0	0	2	0	2	0

Table 3

Discontinuation of subjects on treatment.

Reasons for treatment cessation	Dose Level			
	Level 1 (<i>n</i> = 6)	Level 2 (<i>n</i> = 6)	Level 3 (<i>n</i> = 5)	Level 4 (<i>n</i> = 17)
Disease progression	1	4	0	5
Adverse event	0	1	3	4
Completion of protocol treatment	5	1	2	8